

Research paper

Label-free, real-time monitoring of *IgE*-mediated mast cell activation on microelectronic cell sensor arrays

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Abstract

Immunoglobulin E (*IgE*)-mediated mast cell activation is involved in the immediate phase of allergic reactions and plays a central role in the onslaught and persistence of allergic diseases. *IgE*-mediated mast cell activation includes two important events: cell sensitization resulting from *IgE* binding to Fc (FcεRI) receptor and cell activation triggered by allergen-mediated oligomerization of membrane-bound *IgE*. Real-time monitoring of these events is needed to dissect the molecular mechanisms underlying *IgE*-mediated mast cell activation. Existing technologies are limited to label-based end-point assay formats, which detect either early signaling or final phase of mast cell activation. We describe a microelectronic cell sensor-based technology allowing dynamic monitoring of *IgE*-mediated mast cell sensitization and activation in real-time without any labeling steps. RBL-2H3 mast cells were cultured onto the surface of microelectronic cell sensor arrays integrated into the bottom of microtiter plates, which record electric properties, such as impedance between cell membrane and sensor surface. In the presence of the allergen, dinitrophenyl (DNP)-bovine serum albumin (BSA), anti-DNP *IgE*-sensitized cells were activated within 5 min and the entire activation process was quantitatively and continuously recorded. Impedance measurements correlate with morphological dynamics and mediator release as measured by β-hexosaminidase activity, and can be blocked by pharmacological agents, inhibiting *IgE*-mediated signaling. The assay on microelectronic cell sensor arrays can be scaled up for high-throughput screening of pharmacological inhibitors of *IgE*-mediated mast cell activation and other cell-based receptor–ligand assays.

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Keywords: Real-time; Label-free; Assay; Mast cell; High-throughput

Abbreviations: *IgE*, immunoglobulin E; DNP, dinitrophenyl; BSA, bovine serum albumin; RT-CES™, Real-Time Cell Electronic Sensing.

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1. Introduction

Allergic diseases, also commonly known as immediate hypersensitivity disorder, are the most common dysfunction of the immune system afflicting 20% of all individuals in the United States.

Allergic diseases can be disabling and potentially fatal and the underlying causes and agents that lead to an allergic reaction are highly complex and heterogeneous. Due to the severity of the allergic diseases, the lack of adequate treatment and the high percentage of the population suffering from various forms of this condition, the pharmaceutical industry has taken a keen interest in developing novel drugs to effectively treat and combat the symptoms of this potentially life threatening disorder.

The primary cells of the immune system that are involved in the allergic response are mast cells, basophils and eosinophils (Abbas and Lichtman, 2000). Basophils and eosinophils differentiate in the bone marrow, circulate in the blood and are recruited to the sites of the inflamed tissue in the late-phase of the allergic reactions (Hamid et al., 2003). In contrast, mast cells are normally distributed throughout the connective tissue and are involved in the immediate phase of immunoglobulin E (IgE)-mediated allergic reactions (Gurish and Austen, 2001). The initial encounter of an individual with an allergen leads to the production of IgE, which binds to the high affinity IgE receptor (FcεRI) on the surface of mast cells causing sensitization of the mast cells (Gould et al., 2003). Subsequent encounter with the allergen leads to cross-linking of the FcεRI-IgE complex on the surface of mast cells triggering a sequence of intracellular events, collectively referred to as mast cell activation, which culminates in the extracellular release of potent inflammatory mediators, many of which are stored in the secretory granules (Corry and Kheradmand, 1999; Kawakami and Galli, 2002). Mast cell activation can be divided into an interdependent early and late phase. The early phase of mast cell activation include phosphorylation and activation of protein tyrosine kinases and their substrates, generation of the second messengers inositol-tris phosphate (IP₃) and diacylglycerol (DAG) and elevation of intracellular calcium levels (Turner and Kinet, 1999; Rivera, 2002). The late phase of mast cell activation includes fusion of secretory vesicles with the membrane, dramatic morphological changes due to remodeling of the actin cytoskeleton, gene expression leading to the synthesis and secretion of potent inflammatory cytokines and synthesis of lipid mediators that have a variety of effects on blood vessels, bronchial

smooth muscle and leukocytes (Corry and Kheradmand, 1999).

Based on the various steps involved in the initiation and execution of the immediate hypersensitivity response, there are multiple potential targets for pharmaceutical intervention (Marone et al., 2002). The elucidation of the intrinsic signaling pathways underlying IgE-mediated mast cell activation together with the advent of combinatorial chemistry provide ample opportunity to employ small molecular inhibitors to target key proteins and enzymes involved in mast cell activation (Marone et al., 2002). Hence, there is an urgent need for high-throughput molecular and cellular assays to screen for potential modulators of these signaling pathways. With regards to IgE-mediated signaling, the current assays are single point assays or endpoint assays, which measure the cumulative release of these mediators and also involve utilization of labels and manipulation of the cells, such as fixation or lysis. The fact that these are non-homogenous single point assays utilizing expensive reagents such as antibodies and cellular manipulation does not warrant adaptability for high-throughput analysis that is required to screen large chemical libraries.

In this paper, we describe a novel assay which is aimed at addressing the limitations in current methods and technologies for assaying IgE-mediated mast cell activation by utilizing electronic measurement and sensing of cells. This approach is based on the measurement of cell-electrode impedance and it integrates cell biology with microelectronics for electronic detection of biological processes. For measurement of cell-electrode impedance using Real-Time Cell Electronic Sensing (RT-CES™, <http://www.aceabio.com>) technology, microelectrodes having appropriate geometries are fabricated onto the bottom surfaces of microtiter plate facing into the wells. Cells are introduced into the wells of the devices and make contact with and attach to the electrode surfaces. The presence, absence or change of properties of cells such as cell number, cell morphology and adhesive properties of the cells affects the electronic and ionic passage on the electrode sensor surfaces. Measuring the impedance between or among electrodes provides important information about biological status

of cells present on the sensors. Analog electronic readout signals are measured automatically and in real time, and are converted to digital signals for processing and analysis.

2. Materials and methods

2.1. Cells and reagents

The RBL-2H3 cell line was purchased from American Type Culture Collection (ATCC) and maintained in DMEM containing 10% fetal bovine serum at 37 °C and 5% CO₂. Mouse monoclonal anti-dinitrophenyl IgE antibody (Clone SPE-7) and dinitrophenyl (DNP)-HSA were purchased from Sigma Aldrich (St. Louis, MO). The Src-specific inhibitor SU6656, MEK-specific inhibitor PD98059 and the PLC-specific inhibitor U73122 were purchased from Calbiochem (La Jolla, CA). The PKC-specific inhibitor bisindolylmaleimide and the Syk inhibitor piceatannol were purchased from Sigma. Rhodamine-phalloidin was obtained from Molecular Probes (Eugene, OR). Lab-Tek chamber slides were purchased through VWR Scientific.

2.2. RT-CES measurement of cell-electrode impedance

ACEA RT-CES™ system comprises three components, an electronic sensor analyzer, a device station and 16× microtiter devices. Microelectrode sensor arrays were fabricated on glass slides with lithographical microfabrication methods and the electrode-containing slides are assembled to plastic trays to form electrode-containing wells. The device station receives the 16× microtiter devices and is capable of electronically switching any one of the wells to the sensor analyzer for impedance measurement. In operation, the devices with cells cultured in the wells are connected to device station and placed inside an incubator. Electrical cables connect the device station to the sensor analyzer. Under the RT-CES software control, the sensor analyzer can automatically select wells to be measured and continuously conduct impedance measurements. The impedance data from the analyzer is transferred to a computer, analyzed and processed by the integrated software.

Impedance measured between electrodes in an individual well depends on electrode geometry, ionic concentration in the well and whether there are cells attached to the electrodes. In the absence of the cells, electrode impedance is mainly determined by the ion environment both at the electrode/solution interface and in the bulk solution. In the presence of the cells, cells attached to the electrode sensor surfaces will alter the local ionic environment at the electrode/solution interface, leading to an increase in the impedance. The more cells there are on the electrodes, the larger the increase in cell-electrode impedance. Furthermore, the impedance change also depends on cell morphology and the extent to which cells attach to the electrodes.

To quantify cell status based on the measured cell-electrode impedance, a parameter termed Cell Index is derived, according to

$$CI = \max_{i=1,\dots,N} \left(\frac{R_{\text{cell}}(f_i)}{R_b(f_i)} - 1 \right)$$

where $R_b(f)$ and $R_{\text{cell}}(f)$ are the frequency-dependent electrode resistances (a component of impedance) without cells or with cell present, respectively. N is the number of the frequency points at which the impedance is measured. Thus, Cell Index is a quantitative measure of the status of the cells in an electrode-containing well. Under the same physiological conditions, more cells attached onto the electrodes leads to larger $R_{\text{cell}}(f)$ value, leading to a larger value for Cell Index. Furthermore, for the same number of cells present in the well, a change in the cell status such as morphology will lead to a change in the Cell Index. For example, an increase in cell adhesion or cell spreading leads to larger cell-electrode contact area which will lead to an increase in $R_{\text{cell}}(f)$ and thus a larger value for Cell Index.

Twenty thousand RBL-2H3 cells were seeded per well of 16× microtiter device and monitored by the RT-CES™ system. The cells were allowed to attach and spread for 5–24 h prior to the addition of IgE or 4β-phorbol 12 myristate 13 acetate (PMA) at the indicated final concentration. The cell-electrode impedance was continuously measured and the corresponding, time-dependent Cell Index values were derived and recorded.

2.3. Fluorescence microscopy

RBL-2H3 cells were seeded in 16-well Lab-Tec chamber slides and allowed to attach and spread for 24 h. The cells were stimulated with anti-DNP IgE at a final concentration of 100 ng/ml or a non-specific mouse IgG at 100 ng/ml, and then 16 h later the media was aspirated, replaced with fresh media and treated with 100 ng/ml DNP-bovine serum albumin (BSA) for the indicated time and then fixed with 4% paraformaldehyde. The cells were washed 3× with PBS, permeabilized in PBS containing 0.2% TX-100 and blocked in PBS containing 0.5% BSA. The cells were then stained with rhodamine-phalloidin for 30 min, washed 3× with PBS and visualized and imaged using the tritc filter on a Nikon E400 epi-fluorescence microscope and Nikon ACT software.

2.4. β -Hexosaminidase assay

IgE-sensitized RBL-2H3 cells growing in 96-well plates were washed and incubated in Tyrode buffer (10 mM Hepes, pH 7.4, 130 mM NaCl, 5 mM KCl, 1.4 mM CaCl_2 , 1 mM MgCl_2 , 5.6 mM glucose and 0.1% BSA) and stimulated with 100 ng/ml DNP-BSA. After 1 h, the supernatant was removed and the cell monolayer was lysed in Tyrode buffer containing 0.5% TX-100 to obtain total cellular β -hexosaminidase activity. β -hexosaminidase activity was measured in both supernatant and the cell monolayer using the substrate 4-nitrophenyl-2-acetamido-2-deoxy- β -D-glucopyranoside (1 mg/ml). After 1 h incubation at 37 °C, the reaction was stopped by the addition of 2 volumes of 0.4 M glycine pH 10.7. The absorbance at 405 nm was read in Molecular Devices ELISA reader. β -Hexosaminidase activity in the supernatant was displayed as percent of total activity.

3. Results

3.1. RT-CES measurement of RBL-2H3 mast cell activation

IgE-mediated RBL-2H3 mast cell activation in the presence of antigen leads to initiation of signaling cascade resulting in degranulation of secretory vesicles, which contain mediators of allergic reaction

such as histamine. In addition, IgE-mediated stimulation through Fc ϵ RI also leads to dramatic remodeling of the actin cytoskeleton (Oliver et al., 1997). Since monitoring of cell-electrode impedance provides information about the parameters of cell morphology and adhesion, we sought to determine the cell-electrode impedance response of RBL-2H3 mast cells that were pre-sensitized with IgE in the presence of antigen application.

RBL-2H3 mast cells were seeded onto the surface of ACEA's 16× microtiter plates with integrated microelectronic sensor arrays in the bottom of each well. The cells were allowed to adhere to the surface of the sensors and 18 h later were sensitized with anti-DNP IgE (Fig. 1). Approximately, 24 h later, DNP-BSA at a final concentration of 100 ng/ml was applied to the cells to induce oligomerization of the IgE-bound Fc ϵ RI receptor and induce mast cell activation. The cell-electrode impedance measurements were continuously monitored using the RT-CES™ system. As shown in Fig. 1, treatment with 100 ng/ml anti-DNP-IgE leads to a slight but reproducible change in cell-electrode impedance response. DNP-BSA appli-

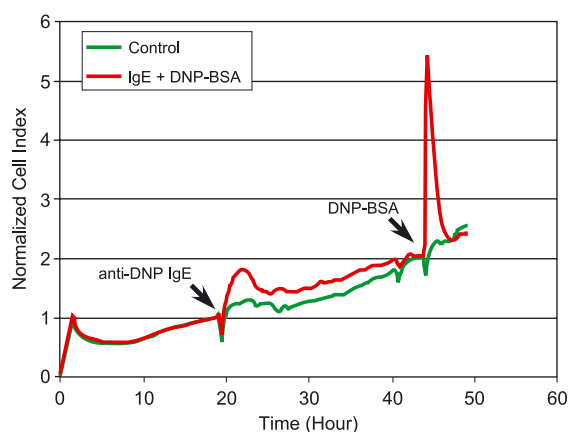


Fig. 1. Cell-electrode impedance detection of RBL-2H3 mast cells sensitized with anti-DNP IgE and activated by the application of DNP-BSA. RBL-2H3 mast cells were seeded at a density of 20,000 cells/well onto the surface of ACEA's 16× microtiter device, which is integrated with microelectronic sensor arrays and the impedance was continuously measured every 30 min and displayed as Cell Index (see Section 2) using the RT-CES™ system. Approximately 18 h after seeding, the cells were incubated with 100 ng/ml anti-DNP IgE followed by application of 100 ng/ml DNP-BSA 24 h later. Impedance measurements, displayed as Cell Index, were performed at 5-min intervals post-DNP-BSA application.

cation induced an immediate and transient increase in the impedance value, which was detectable within 5 min of DNP-BSA application, maximal by 30 min and returned to baseline in approximately 4 h. IgE-mediated activation of mast cells not only lead to dramatic morphological changes (Pfeiffer et al., 1985) but also augmentation of integrin-mediated cell adhesion (Wyczolkowska et al., 1994), both of which contribute to cell-electrode impedance measurements using the RT-CES™ system.

3.2. RT-CES™ measurement of mast cell activation correlates with cytoskeletal dynamics and degranulation

To determine if the IgE-mediated cell-electrode impedance increase correlates with RBL-2H3 mast cell activation, both IgE-mediated morphological dynamics and mediator release were monitored.

RBL-2H3 mast cells were sensitized and activated as described above and at the indicated time points, fixed with paraformaldehyde and stained with rhodamine-phalloidin to visualize the actin cytoskeleton (Fig. 2A). As seen in Fig. 2A and shown previously (Pfeiffer et al., 1985; O'Lunaigh et al., 2002; Powner et al., 2002), DNP-BSA-mediated cross-linking of IgE-bound FcεRI receptor leads to time-dependent remodeling of the actin cytoskeleton. The cells undergo extensive ruffling which is apparent as early as 2.5 min post-DNP-BSA addition, followed by morphological changes, which lead to cell spreading and formation of lamellapodia. The peak cytoskeletal reorganization is observed at 30–45 min post-IgE stimulation which correlates directly with the peak cell-electrode impedance response using the RT-CES™ system and returns to spindle shape morphology 4 h later. As a control, RBL-2H3 mast cells were also sensitized with an irrelevant IgG and subsequently cross-linked with DNP-BSA. No obvious cytoskeletal and morphological changes were observed (data not shown).

As an additional marker for RBL-2H3 mast cell activation, β-hexosaminidase activity was also measured in response to IgE stimulation in the presence of antigen cross-linking. The enzyme β-hexosaminidase is stored within the secretory vesicles and is a marker for mast cell degranulation. It has been shown that β-hexosaminidase is released into the culture media in

response to antigen-mediated cross-linking of IgE-bound FcεRI on the surface of mast cells (Razin et al., 1983). RBL-2H3 cells were sensitized with anti-DNP IgE, activated by application of DNP-BSA and β-hexosaminidase activity was measured as described in materials and methods section. Antigen cross-linking leads to two and a half to four-fold increase in β-hexosaminidase activity depending on the experiment (Fig. 2B). Taken together, IgE-mediated mast cell-electrode impedance correlates directly with morphological changes and degranulation, which is characteristic of mast cell activation. Therefore, cell-electrode impedance measurements can be used as readout for mast cell activation.

3.3. Comparison of PMA and IgE-mediated morphological dynamics using the RT-CES™ system

PMA has been shown to induce morphological changes such as membrane ruffling and cell spreading of RBL-2H3 cells in the absence of degranulation (Ludowyke et al., 1994; Kawasugi et al., 1995; Nakamura et al., 1996). Therefore, we were interested in comparing the dynamics of PMA induced morphological changes with that of antigen-mediated morphological changes which is accompanied by degranulation, using the RT-CES system.

RBL-2H3 cells were seeded in ACEA's 16× microtiter plate and the cells were monitored continuously using the RT-CES™ system. At approximately 48 h after seeding, the cells were stimulated with 20 ng/ml of PMA. In addition, in another well, cells were sensitized with anti-DNP IgE at 20 h after seeding and treated with DNP-BSA at the same time as PMA addition. PMA induces an immediate increase in cell index to about four-fold above basal level at peak amplitude and the duration of which lasts approximately 20 h (Fig. 3 and Table 1). On the other hand, antigen stimulation of IgE-sensitized cells reproducibly and characteristically leads to about 2.5-fold increase in cell index at about 30 min post-DNP-BSA addition with a total duration of about 4 h. In summary, even though PMA induces transient impedance changes in RBL-2H3 cells, the kinetics of the cell-electrode impedance response varies considerably when compared to antigen-mediated-response with regards to peak amplitude,

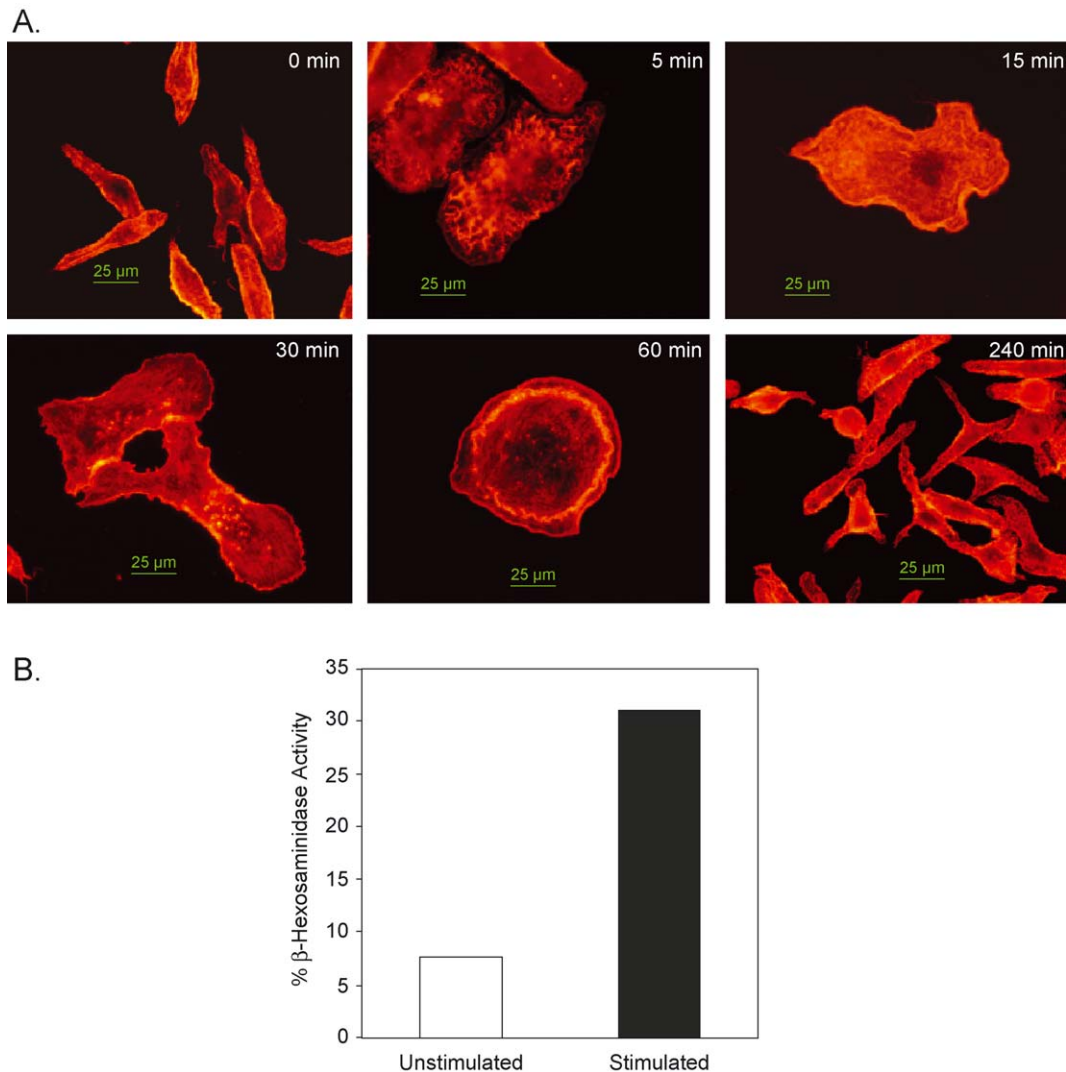


Fig. 2. Correlation between RBL-2H3 mast cell morphological dynamics and mediator release. (A) RBL-2H3 mast cells were sensitized with 100 ng/ml IgE or treated with a control IgG antibody and subsequently activated with DNP-BSA. The cells were fixed with paraformaldehyde at the indicated time points, permeabilized and stained with rhodamine-phalloidin. The cells were visualized and photographed with a Nikon E-400 immunofluorescence microscope equipped with a CCD camera. (B) RBL-2H3 cells were sensitized with IgE, activated by the addition of DNP-BSA. β -hexosaminidase activity was measured as described in Section 2.

the time it takes to reach peak amplitude and the duration of the response (Table 1).

3.4. Pharmacological inhibition of mast cell activation as detected by the RT-CESTM system

Antigen-mediated aggregation of the IgE-bound Fc ϵ RI triggers a signaling cascade which involves a

number of signaling proteins such as protein kinases, protein phosphatases and phospholipases amongst others whose activity and function is indispensable for mast cell activation (Turner and Kinet, 1999). Accordingly, we were interested in determining the effect of pharmacological inhibitors of some of these signaling proteins on mast cell activation as monitored by cell-electrode impedance measurements.

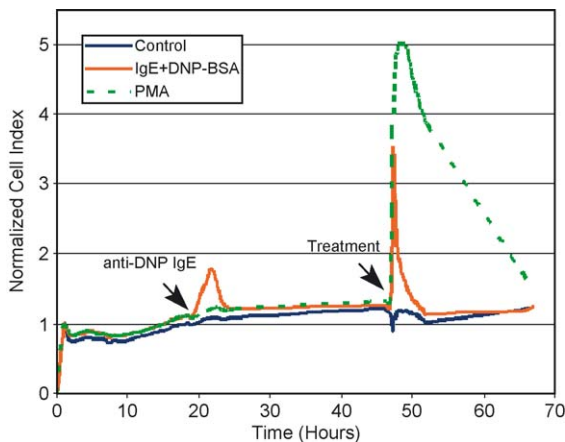


Fig. 3. Comparison of PMA and *IgE*-DNP-BSA-mediated cell-electrode impedance response using the RT-CES™. RBL-2H3 cells seeded on microelectronic sensor arrays were left untreated or treated with *IgE* and DNP-BSA or with 20 ng/ml PMA. The impedance values were continuously monitored and displayed as Cell Index using the RT-CES™ system.

RBL-2H3 cells seeded in 16× microtiter plate device and pre-stimulated with anti-DNP-*IgE* were incubated for 1 h with the indicated doses of the PKC-specific inhibitor bisindolylmaleimide and subsequently treated with 100 ng/ml DNP-BSA (Fig. 4). As shown in Fig. 4, bisindolylmaleimide inhibited mast cell activation in a concentration-dependent manner. A number of other pharmacological agents such as SU6656, an inhibitor of Src family kinases, U73122, an inhibitor of phospholipase C, and picea-

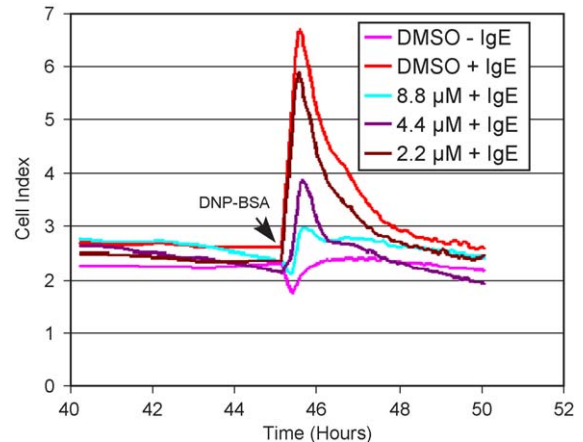


Fig. 4. Monitoring of the inhibitory effect of PKC inhibitor, bisindolylmaleimide, on mast cell activation by RT-CES™. RBL-2H3 mast cells were sensitized with 100 ng/ml anti-DNP-*IgE* and then incubated with the indicated concentrations of the bisindolylmaleimide 1 h prior to addition of 100 ng/ml DNP-BSA. The impedance value, displayed as Cell Index, was monitored using the RT-CES™ system.

Table 1
Comparison of the kinetics of cell-electrode impedance response of RBL-2H3 mast cells treated with *IgE*/DNP-BSA or with PMA

Treatment	Peak amplitude ^a	Time to peak amplitude ^b (min)	Duration of response ^c (min)
<i>IgE</i> +DNP-BSA (<i>N</i> =5)	2.7±0.26	33±5.7	234±31
PMA (<i>N</i> =4)	3.85±0.05	87.2±3.51	1170±49

N=number of experiment carried out separately.

^a Peak amplitude is defined as the maximum cell-electrode impedance response divided by baseline cell-electrode impedance prior to treatment. Data are expressed as mean±standard deviation.

^b Time to peak amplitude is defined as the increment of time in minutes it takes after treatment to reach peak cell-electrode impedance value.

^c Duration of the response is defined as the increment of time in minutes between the initiation of the change in cell-electrode impedance response after treatment to the point where the response returns to baseline.

tannol, an inhibitor for Syk tyrosine kinase, were also tested. The IC-50 value for these pharmacological inhibitors at peak response was calculated and is shown in Table 2. All inhibitors tested dose-dependently inhibited antigen-mediated RBL-2H3 mast cell activation as measured by RT-CES™ system. Furthermore, as a comparison, we also determined the IC-50 values of these pharmacological inhibitors using the standard β-hexosaminidase activity assay (Table 2). In general, there is good concurrence between the IC-50 values determined using the RT-CES™ system and β-hexosaminidase assay. These findings are in agreement with previously published data indicating that the Src, PLC, PKC and Syk inhibitors completely block *IgE*-mediated mast cell degranulation and activation (Oliver et al., 1994; Amoui et al., 1997; Moriya et al., 1997; Tedeschi et al., 2000). In summary, the results presented here indicate that real-time monitoring of *IgE*-mediated mast cell activation on microelectronic cell sensor arrays offer a convenient way of assessing mast cell activation. The assay does not require any cellular manipulation such as labeling, fixation or lysis. The assay was validated by demonstrating that the cell-electrode impedance measurement correlates directly with *IgE*-mediated mast cell activation as measured by actin cytoskeleton dynamics and mediator release. Furthermore, previously characterized

Table 2

Comparison of the IC-50 values of different pharmacological inhibitors of mast cell activation using RT-CES™ system and β -hexosaminidase assays

Compound	Target	IC-50 (μ M) β -hexosaminidase	IC-50 (μ M) RT-CES
Bisindolylmaleimide	Protein Kinase C	2.5 $\pm$ 1 (N=3)	2.9 $\pm$ 1.7 (N=3)
SU 6656	SRC	1.25 $\pm$ 0.1 (N=3)	5.8 $\pm$ 3.5 (N=5)
U 73122	Phospholipase C	0.63 $\pm$ 0.2 (N=3)	0.93 $\pm$ 0.6 (N=5)
Piceatannol	Syk	2 $\pm$ 0.5 (N=3)	1 (N=2)

RBL-2H3 cells were seeded on ACEA's 16X microtiter plates, sensitized with 100 ng/ml anti-DNP IgE and incubated with increasing concentrations of the indicated inhibitors 1 h prior to the addition of 100 ng/ml DNP-BSA. The IC-50 $\pm$ standard deviation (S.D.) value was determined using the peak amplitude value for each concentration of the inhibitor. For IC-50 determination using β -hexosaminidase activity, RBL-2H3 cells were seeded in 96-well tissue culture plates, sensitized with 100 ng/ml anti-DNP IgE. One hour prior to DNP-BSA addition, the media was removed, replaced with Tyrode buffer and incubated with increasing concentration of the indicated inhibitors. β -Hexosaminidase activity was determined as described in Section 2.

specific inhibitors of mast cell activation pathway inhibit mast cell activation as measured by the RT-CES™ system with similar potency, further validating this assay.

4. Discussion

Allergic diseases continue to pose a formidable economic and medical challenge to industrialized societies. The prevalence of allergic diseases has seen an upward trend in recent years and the economic toll has been significant due to lost work days and medical costs. Most of the current therapies for various allergic conditions are aimed at alleviating the symptoms of the disease rather than directly addressing and treating the underlying cause. While there are multiple venues to focus on for novel anti-allergic therapies, one promising area of research has been to employ small chemical inhibitors to target key proteins and enzymes in IgE-mediated mast cell activation pathway, which plays a central role in the onset of allergic reactions (Marone et al., 2002; Holgate and Broide, 2003). The results presented in this manuscript describe a novel assay platform for IgE-mediated mast cell activation that is based on measurement of impedance of RBL-2H3 mast cells cultured on microelectronic sensor arrays. IgE-mediated mast cell-electrode impedance response correlates with mast cell activation events such as cytoskeletal rearrangement and mediator release. Furthermore, previously characterized pharmacological inhibitors which inhibit mast cell activation also inhibit IgE-mediated mast cell-electrode impedance response.

The current technologies assaying for mast cell activation are mainly composed of enzymatic assays, fluorescent-based assays and ELISA. The enzyme-based assays involve measurement of the activity of key proteases such as chymase, tryptase or β -hexosaminidase, which themselves are mediators of the allergic reaction (Razin et al., 1983; Dietze et al., 1990; Lavens et al., 1993; Satomura et al., 2002). The activity of these mediators are measured in the supernatant and displayed as fraction of total enzyme activity present in the cells. A fluorescent-based assay for mast cell activation which involves fixation and specific labeling of activated mast cells with fluorescently-conjugated annexin-V and detection by flow cytometry has also been described (Demo et al., 1999). Rearrangement of membrane-bound phospholipids due to mast cell degranulation allows for the specific labeling of phosphatidylserine membrane lipids by annexin-V. An additional assay for mast cell activation is quantification of mediator release in the supernatant by immunological methods. Kits are available commercially which measures and quantifies the cumulative release of mediators such as histamine, TNF, interleukins and other mediators. While all of the above mentioned assays can be informative with small sample sizes they involve extensive labeling steps, washing steps and lysing and fixation steps, which are not amenable for the kind of high-throughput analysis that is required to screen large chemical libraries. The IgE-mediated mast cell activation assay described here does not require any labeling, fixation and cell lysis. Therefore, the lack of cellular manipulations combined with real-time monitoring of IgE-mediated sensitization and activation

makes it an ideal assay for monitoring mast cell activation. Furthermore, the assay is readily adaptable to 96-well microtiter plate format or 384-well plate format which is convenient for high-throughput analysis of chemical compound libraries.

The use of electronic impedance detection technology to monitor biological processes was first reported by Giaever and Keese (1984). Early work has demonstrated that electronic impedance detection can be used to non-invasively and continuously monitor cellular events such as cell adhesion and spreading, cellular micromotion, cell migration due to wound healing and morphological changes due to actin cytoskeleton rearrangement (Giaever and Keese, 1984, 1986, 1991, 1993). Specifically, electronic impedance technology has been used to monitor the attachment and spreading of MDCK cells on sensor surfaces coated with different extracellular matrix material (Lo et al., 1999). Also, endothelial cell and fibroblast shape changes due to cytoskeletal rearrangement has been monitored by electronic impedance technology (Smith et al., 1994; Wang et al., 1995).

Cell-electrode impedance reading is primarily influenced by three main parameters: the number of cells seeded on microelectronic sensor arrays, the shape of the cell and the strength of cell adhesion to the electrode surface. *IgE*-mediated RBL-2H3 mast cell activation is accompanied by an increase in effective surface area due to fusion of secretory granules, dramatic actin cytoskeleton rearrangement as well as an increase in integrin-mediated adhesion (Pfeiffer et al., 1985; Wyczolkowska et al., 1994). Morphological dynamics combined with an increase in adhesive interaction of the cell with the electrode surface leads to an increase in antigen-dependent *IgE*-mediated mast cell electrode impedance value. Furthermore, pharmacological inhibitors of signaling proteins which participate in *IgE*-mediated mast cell activation inhibit *IgE*-mediated mast cell activation in a dose-dependent manner (Fig. 4 and Table 2). Pharmacological inhibitors of Src, Syk, PLC γ and PKC led to a dose-dependent inhibition of *IgE*-mediated RBL-2H3 mast cell activation as monitored by cell-electrode impedance. Moreover, the IC-50 values obtained for each of these pharmacological inhibitors using the RT-CESTM system are corroborated by using the traditional mediator release assay (Table 2). Utilization of these pharmacological inhibitors further validates this assay

and indicates that the RT-CESTM system can be used to assess mast cell activation.

It is important to note that changes in RBL-2H3 morphological dynamics are not always reflective of the activation state of the cell leading to degranulation and mediator release. For example, treatment of RBL-2H3 mast cells with PMA, which is an activator of PKC, leads to dramatic morphological changes such as membrane ruffling and lamellapodia formation in the absence of degranulation and mediator release (Ludowyke et al., 1994; Kawasaki et al., 1995; Nakamura et al., 1996). Furthermore, treatment of RBL-2H3 mast cells with the cytoskeletal disrupting agent, cytochalasin B actually promotes *IgE*-mediated RBL-2H3 mediator release (Pierini et al., 1997; Frigeri and Apgar, 1999). We compared PMA and antigen-mediated cell-electrode impedance response of RBL-2H3 mast cells. PMA induced a dramatic increase in cell-electrode impedance response which is reflective of the morphological status of the cell. However, when compared to *IgE*-mediated response, the dynamics of the PMA response was significantly different with regards to the duration of the response, peak amplitude response and the time needed to reach peak amplitude (Fig. 3 and Table 1). Thus, even though both treatments leads to morphological and adhesive changes, the kinetics combined with the extent of the response clearly distinguishes the effect of PMA, which is not accompanied by mediator release, and antigen-mediated response, which is accompanied by degranulation and mediator release. Therefore, each treatment has its own characteristic “signature” pattern, which is obtained as a result of real-time monitoring. Furthermore, since RT-CES monitoring of RBL-2H3 mast cell activation relies on cytoskeletal and morphological dynamics, any compound that affects the actin-cytoskeleton and not necessarily participate in *IgE*-mediated signaling will most likely register as a false positive in this assay. Therefore, as with any cell-based assay, it is imperative to confirm the candidate compounds or “hits” with other assays for *IgE*-mediated mast cell activation.

In summary, the real-time and label-free *IgE*-mediated mast cell activation assay described in this manuscript should provide researchers in pharmaceutical industry as well as academia with a new and convenient tool to assess and quantify *IgE*-mediated mast cell activation. The adaptability of this assay into

96× microtiter plates with microelectronic sensors integrated in the bottom of the wells makes it ideal for high throughput analysis to screen large chemical compound libraries. Furthermore, microelectronic cell sensor technology in general can be used to assess other receptor ligand interactions.

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